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For: "CELL SUSPENSION PREPARATION
TECHNIQUE AND DEVICE"

Group Art Unit: 1743

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CLAIM FOR PRIORITY UNDER 35 U.S.C. § 119(b)Commissioner for Patents
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May 13, 2002

Sir:

In accordance with the provisions of 35 U.S.C. § 119(b) and 37 C.F.R. § 1.55(a), applicant hereby claims priority from Australian Provisional Application No. PR 2989, filed February 7, 2001.

Also enclosed is a certified copy of the Australian Provisional Application. Applicant respectfully requests that the enclosed application be made of record as the foreign priority document for the pending application, under 35 U.S.C. § 119(b).



No fee is believed due. However, the Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date indicated below.

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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 2989 for a patent by CLINICAL CELL CULTURE PTY LTD filed on 7 February 2001.

I further certify that the name of the applicant was amended to CLINICAL CELL CULTURE PTY LTD and METROPOLITAN HEALTH SERVICES BOARD pursuant to the provisions of Section 104 of the Patents Act 1990.

I further certify that the name of the applicant was amended to CLINICAL CELL CULTURE PTY LTD and MCCOMB FOUNDATION INC. pursuant to the provisions of Section 104 of the Patents Act 1990.

I further certify that the above application is now proceeding in the name of MCCOMB FOUNDATION INC. pursuant to the provisions of Section 113 of the Patents Act 1990.

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PROVISIONAL SPECIFICATION

Invention Title: "Cell Suspension Preparation Technique and Device"

The invention is described in the following statement:

"CELL SUSPENSION PREPARATION TECHNIQUE AND DEVICE"

FIELD OF THE INVENTION

This invention relates to a simple, rapid and cost effective technique for grafting of cells, and in particular to a device for preparing a suspension of cells from a tissue sample obtained from a donor site and applying that suspension of cells to a recipient site.

BACKGROUND ART

There are many methods of treating wounds known to those skilled in the art. For example, skin grafting techniques exist which aim to reconstruct the skin covering areas of the body where there is either damage or defects to the skin. In general, these types of grafts are classified according to their host-donor relationship and by their thickness. The most clinically applied graft is the autogenous graft, whereby tissue is taken from one area of the body and applied to another area. The grafted tissue then develops a new blood supply and attaches to the underlying tissues.

There are several types of skin grafts presently used, including split-thickness, full-thickness grafts, and micro-grafting. Each of these graft types must be prepared using certain techniques, and each one has its inherent advantages and disadvantages. Split-thickness grafts often require considerable skill, time and expensive equipment to perform. Further, donor sites are painful, result in scarring and limit the area able to be covered. Although they may be more successful than full-thickness grafts, they are usually less cosmetically attractive. Full-thickness grafts require less skill or expensive equipment, and their cosmetic appearance is better than that of split-thickness grafts. However, Full-thickness grafts do not "take" as well as split-thickness grafts. Micro-grafts are more easily accomplished and require no special instruments. However, their cosmetic appearance is not as good as other techniques, as the resulting scarring is unacceptable.

A variation to the above grafting techniques is the mesh graft, which is a type of split-thickness or full-thickness skin graft in which parallel rows of slits are cut in

the tissue being treated. Some of the advantages of mesh grafts include: a greater coverage of the effected area, drainage of blood or serum from under the graft, and increased conformity of the graft to uneven recipient areas. This technique has been very successful, with 90 to 100 per cent "take" when the 5 grafts have been applied on healthy granulation beds.

An alternative to split-skin grafting is to form a blister under suction at a donor site and transplant to the recipient site. The production of blisters to treat wounds has been used since the 1960s. The blisters are produced by a suction device, such as Dermavac™, at a suction pressure of approximately 250-300 mmHg for 1-2 10 hours. The blisters are then cut off and placed on the wound. The healing time is around 10-14 days. There are several disadvantages of this method such as the amount of time required to prepare the graft is too long and the graft may not result in re-pigmentation of the area; or uneven pigmentation is common around the edges of the area of treatment.

15 Micro-grafting has become a more common approach for large area cover and involves the "snipping off" of a number of very small sections of tissue from a donor site and applying then to a dressing, which is in turn applied to the wound area.

The most advanced technology for the generation of a tissue *in vitro* is to culture 20 epidermis. Cultured epithelial autografts (CEA) are an important adjunct in the coverage of burns and other situations in which large areas of the body's surface experience skin loss. There are many centres throughout the world with tissue culture facilities whose aim is to produce autologous epithelial grafts for use in a wide variety of applications. The usefulness and application of CEA is related to 25 its ability to achieve confluent cells sheets suitable for grafting. This technique overcomes many of the disadvantages of the previous treatments described above. For example, cultured epithelial autografts reduce the demand for donor sites. However, these autografts are slow growing and require time for culturing of the grafts, which often exceeds the time of preparation of the recipient's sites.

30 The present invention provides a cellular suspension together with a method for preparing that suspension and a device for its preparation each of which seek to

ameliorate one or more of the disadvantages associated with prior art grafting technology.

General

Those skilled in the aforementioned art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It should be understood that the invention includes all such variation and modifications.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only.

10 Functionally equivalent products, compositions and where appropriate methods are clearly within the scope of the invention as described herein.

Throughout this specification and the claims that follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group

15 of integers but not the exclusion of any other integer or group of integers.

SUMMARY OF THE INVENTION

The subject invention relates to a unique cell suspension and method for its preparation that is rapid, efficient and simple to prepare and apply. It also relates to a method for treating a patient using the unique cell suspension and to an

20 apparatus suitable for use in the method preparation. Use of the described device while not essential for practising the method of the invention has been found to significantly reduce the complexity associated with the use of conventional grafting technology such as CEA.

According to a first aspect of the invention there is provided a method for

25 preparing a cell suspension suitable for application to a patient, which method comprises the steps of:

(a) subjecting a tissue sample including cells suitable for grafting to a patient, to at least an enzyme suitable for dissociating cohesive pieces of tissue stratum in the sample;

(b) removing the tissue sample from the enzyme solution used in step (a) and harvesting in the presence of a nutrient solution cells from the tissue sample, cells suitable for grafting on to a patient wherein the nutrient solution is (i) free of xenogenic serum, (ii) capable of 5 maintaining the viability of the cells until applied to a patient and (iii) is suitable for direct application to a region on a patient undergoing tissue grafting; and

(c) filtering the cellular suspension produced according to step (b) to remove large cellular conglomerates.

10 In a preferred form of the invention the filtered cellular suspension is diluted to an appropriate cell density using a nutrient solution.

According to a second aspect of the invention there is provided a cell suspension produced according to the above method. Preferably the cells in the suspension are autologous cells (ie they are isolated from the patient requiring an autograft).

15 The inventors have observed that by removing xenogenic serum from the cell suspension there is less likelihood of transmission of infection caused by xenogenic reactions between a patient and the serum. Another feature of the cell suspension produced according to the above method is that the tissue sample used to isolate the cells in the suspension is removed from the enzyme solution 20 before the cells are harvested. When cells are exposed to enzymes capable of damaging inter-cellular adhesion the viability of the cellular suspension decreases over time thereby reducing the efficiency of the grafting when applied to a patient. The cell suspension produced according to the above method has been observed to possess greater cellular viability compared to comparative methods that 25 suspend the cells in the presence of enzymes like trypsin.

According to a third aspect of the invention there is provided a method of treating a patient in need of graft surgery, said method comprising the steps of:

(a) preparing an cell suspension according to the above method;

(b) administering the suspension directly to a region on the patient that 30 requires a cell graft in a manner that facilitates spreading of the cell suspension in a relatively even distribution over the graft region.

In an alternate form, the invention consists in the use of a cellular suspension suitable for grafts, which suspension is prepared according to the following steps:

- (a) subjecting a tissue sample including cells suitable for grafting to a patient, to an enzyme suitable for dissociating cohesive pieces of the tissue stratum in the sample;
- 5 (b) removing the sample from the enzyme solution used in step (a) and harvesting in the presence of a nutrient solution cells from the tissue sample, which cells are suitable for grafting on to a patient wherein the nutrient solution is (i) free of xenogenic serum, (ii) capable of maintaining the viability of the cells until applied to a patient and (iii) is suitable for direct application to a region on a patient undergoing tissue grafting;
- 10 (c) filtering the cellular suspension produced according to step (b) to remove large cellular conglomerates;

15 for the preparation of medicament suitable for the treatment of tissue disorders requiring grafting.

According to a fourth aspect of the invention there is provided an apparatus for developing a tissue regeneration solution, said apparatus comprising:

- (a) a heating means suitable for heating an enzyme solution to a required temperature and which is capable of maintaining that solution at the desired temperature for a suitable amount of time; and
- 20 (b) a filter recess comprising a filter means capable of separating large cellular congregates from a cellular suspension.

When the enzyme solution is placed in contact with the heating means it is
25 preferably heated in a manner that avoids localised heating within the solution.

DETAILED DISCLOSURE OF THE INVENTION

Having regard to the above, this invention provides a unique method and or device suitable for producing a transplantable cellular suspension of living tissue suitable for grafting to a patient. In applying the method and or in using the device

donor tissue is harvested, subjected to an enzyme treatment, cells suitable for grafting back to a patient are collected and dispersed in a solution that is suitable for immediate dispersion over the recipient graft site.

The subject invention has many advantages over the prior art some of which are

5 illustrated in the following paragraphs.

1. It provides a time efficient method for supplying a cellular cover to a tissue in a clinical setting. That is, cells are available when needed at the time of surgery. This is achievable because there is a very short preparation period of the cells, thus allowing grafting to be performed peri-operatively or in the rooms of a 10 specialist physician or General Practitioner.

2. It provides a method and an apparatus which significantly reduces the complexity associated with the use of conventional CEA's and is particularly useful in cases of burn injury that have presented late. In some instances, cells are unavailable at the time of surgery, either due to delayed referral of a patient 15 with an unhealed burn or simply because the time needed for culturing of the grafts had exceeded that for preparation of the recipient would bed. The present invention ameliorates the issue of graft preparation time.

3. It aids in the achievement of rapid cell coverage in areas of injury and donor sites. It provides a means for reducing the size of donor sites and eliminates the 20 use of split skin graft donor sites; improves expansion rate of cell coverage; improves the rate of healing of small burns; is useful for small areas of skin reconstructions, such as scars; and improves scar quality.

4. It ameliorates problems associated with the use of solutions used during conventional tissue culture process. According to the method of preparation and 25 treatment the cells used in a graft are suspended in a nutrient solution free of xenogenic serum. That suspension is then placed directly onto the recipient site.

5. It provides a means for the treatment of various skin disorders or diseases. For example, it may be used for the following: epidermal resurfacing, replacement after skin loss, site match-up during re-pigmentation of an area of skin, treatment 30 of burn wounds, leukoderma, vitiligo, piebaldism, in the treatment of scars- for

example, caused through incorrect wound healing, improper scar direction or scar distortion from wound contraction, acne scars; resurfacing cosmetic dermabrasion, resurfacing after laser treatment and in association with dermal reconstruction.

- 5 6. It provides a means to produce a suspension of cells with increased viability. That is, due to the manner of preparation of the cellular suspension cells such as basal cells, Langerhans cells, fibroblasts, melanocytes and keratinocytes typically have enhanced survival rates. This has the advantage of allowing for the correct re-pigmentation of skin after a skin graft.
- 10 7. It allows faster surgery and healing – thereby reducing trauma for patients during the phase of their medical care.

According to the first aspect of the invention there is provided a method for preparing a cell suspension suitable for use in resurfacing and regeneration of damaged tissue.

- 15 According to this method, tissue (preferably of an autologous nature) is harvested from a patient by means known in the art of tissue grafting. Preferably this is achieved by taking a tissue biopsy. With the harvesting of the biopsy consideration must be given to the depth of the biopsy and the surface area size. The depth and size of the biopsy influence the ease at which the procedure can
- 20 be undertaken and speed with which a patient recovers from the procedure. In a highly preferred form of the invention the donor site should be chosen to appropriately match the recipient site, for example post-auricular for head and neck, thigh for lower limbs, inner-upper-arm for upper limbs, or palm for sole or vice-versa.
- 25 Once a biopsy has been harvested from a patient it is placed in a pre-warmed enzyme solution containing an amount of enzyme capable of dissociating cellular stratum in the tissue sample. Preferably, this is achieved using a trypsin solution, however, any other enzyme such as dispase, collagenase that cause cells to become detached from other cells or from solid surfaces may be used for this
- 30 purpose. The enzyme solution used in the method is preferably calcium and

magnesium free. One such solution is preferably calcium and magnesium ion free phosphate buffered saline.

Where the tissue biopsy is derived from a patient's skin (comprising epithelial-dermal cells) the amount of trypsin that might be used in the method is preferably

5 between about 2.5 and 0.1% trypsin per volume of solution. Desirable the trypsin concentration of the solution is about 1 to 0.25%, with about 0.5% trypsin being most preferred.

The time period over which the tissue sample is subjected to the trypsin solution may vary depending on the size of the biopsy sample taken. Preferably the tissue

10 sample is placed in the presence of the trypsin solution for sufficient time to weaken the cohesive bonding between the tissue stratum. For example, where the tissue sample is taken from a patient's skin the sample might be placed in trypsin for a 5 to 50 minute period. Preferably, the tissue sample is immersed in the trypsin solution for between 10 and 30 minutes with 15 to 20 minutes being

15 optimal for most tissue samples.

After the tissue sample has been immersed in the trypsin solution for an appropriate amount of time, the sample is removed from the trypsin and washed with nutrient solution. Washing the tissue sample may involve either partial or complete immersion of the treated sample in the nutrient solution followed by

20 disposal of the wash solution. Alternatively, and more preferably, the wash solution is dripped on the tissue sample in sufficient volume to remove and or significantly dilute any excess trypsin solution from the surface of the sample. Preferably any dilution that might occur would lead to less than 0.01% trypsin in the nutrient solution.

25 The nutrient solution used in the method should have the characteristics of being (i) free of at least xenogenic serum, (ii) capable of maintaining the viability of the cells until applied to a patient and (iii) is suitable for direct application to a region on a patient undergoing tissue grafting. Preferably the nutrient solution is free of all serum but may contain various salts that resemble the substances found in

30 body fluids; this type of solution is often called physiological saline. Phosphate or other non-toxic substances may also buffer it in order to maintain the pH at

approximately physiological levels. A suitable nutrient solution that is particularly preferred is Hartmann's solution.

After application of the nutrient solution to the tissue sample, the cellular stratum of the sample are separated permitting cells capable of reproduction to be 5 removed from the cellular material and suspended in the nutrient solution. Where the tissue sample is skin the dermis and epidermis are preferably separated to allow access to the dermal-epithelial junction of both surfaces.

Cells capable of reproduction are then removed from the separated stratum by any means known in the art. Preferably, the reproductive cells are scraped off the 10 surface of the stratum using an instrument such as a scalpel. Cells capable of reproduction within the dermal-epithelial junction include but are not limited to basal cells, Langerhans cells, fibroblasts, melanocytes and keratinocytes. Following release of the cells from the tissue sample they are suspended in the nutrient solution. Preferably only a small volume of nutrient solution is applied to 15 the tissue sample during this harvesting step otherwise the suspension may become too fluid therein providing difficulties in applying the suspension to the graft.

To avoid excessively large cellular congregates in the cellular suspension the suspension is preferably filtered. Any filter capable of separating excessively 20 large cellular congregates from the suspension may be used in this preferred step of the invention. In a highly preferred form of the invention the filter size is between 0.7 μ m and 0.15 μ m. More preferably it is between 0.5 μ m and 0.2 μ m, with 0.22 μ m being one specific example.

Prior to application to the graft or immediately after filtering, the cellular 25 suspension may be diluted to produce an appropriate cell density suitable for the purpose to which the suspension is to be used.

According to the second aspect of the invention there is provided an aqueous cell suspension produced by the method described in the first aspect of the invention. The cell suspension provided by this method is highly suitable for tissue 30 regeneration and grafting techniques. An important advantage of utilising such a

suspension in grafting technology is that it can be used to greatly expand the area or volume of a wound that can be treated quickly by *in situ* multiplication of a limited number of cells. The number and concentration of cells seeded onto graft site may be varied by modifying the concentration of cells in suspension, or by

5 modifying the quantity of suspension that is distributed onto a given area or volume of the graft site.

By suspending cells in a nutrient solution which is at least (i) free of xenogenic serum, (ii) capable of maintaining the viability of the cells until applied to a patient and (iii) is suitable for direct application to a region on a patient undergoing tissue

10 grafting, the inventors have found that the success rates of patient grafts increases. A partial explanation for this appears to be attributable to the removal of xenogenic serum and more preferably all serum from the cell suspension. Serum is a common additive in grafting culture medium and is well known to cause potential infective problems. Such serum is however generally required for

15 the *in vitro* expansion of the cells. The nutrient solution used in the present invention does not require such serum because the cell population within the suspension is not expanded prior to application to the graft site. Rather cellular multiplication is encouraged on the patient rather than in an *in vitro* system.

Another unique feature of the cell suspension produced according to the method

20 of the first aspect of the invention is that the graft cells are more viable compared to prior art cellular preparation. One possible explanation for this is that the cellular suspension produced from the first aspect of the invention is prepared in nutrient solution as distinct from trypsin solution, which contains powerful digestive enzymes. When cells are exposed to such enzymes for excessive

25 periods of time the viability of the cellular suspension decreases reducing the efficiency of the graft when applied to a patient.

According to the third aspect of the invention there is provided a method of treatment of the patient requiring a tissue graft. By this method the cellular suspension produced according to the first aspect of the invention is applied to a

30 graft site. A liquid suspension containing cells may be manually distributed onto the graft site by any of several techniques, which include spraying, spreading, pipetting and painting.

In a highly preferred form of the invention the suspension is sprayed on to graft site. The suspension may be sprayed through any type of nozzle that transforms liquid into small airborne droplets. This embodiment is subject to two constraints. First, it must not subject the cells in solution to shearing forces or pressures that would damage or kill substantial numbers of cells. Second, it should not require that the cellular suspension be mixed with a propellant fluid that is toxic or detrimental to cells or wound beds. A variety of nozzles that are commonly available satisfy both constraints. Such nozzles may be connected in any conventional way to a reservoir that contains the cellular suspension.

10 Alternatively the suspension may be delivered via a pipette, common "eye-droppers," and or other similar devices to place small quantities of cellular suspension on a graft site.

After the cell suspension has been applied to the recipient graft site, the wound may be dressed with a wound dressing. In a preferred embodiment the dressing 15 is a Surfaseal™ woven nylon dressing. Preferably, the healing of the wound is followed up by standard protocols for skin graft treatment known to those skilled in the art.

According to the fourth aspect of the invention there is provided an apparatus for developing a tissue regeneration solution, which has a heating means suitable for 20 heating an enzyme solution to a required temperature and which is capable of maintaining that solution at the desired temperature for a suitable amount of time; and a filter recess comprising a filter means capable of separating large cellular congregates from a cellular suspension.

In a preferred form of the fourth aspect of the invention the apparatus also 25 includes a reservoir capable of holding a tissue sample and a nutrient solution which solution is also capable maintaining the viability of the cells in the tissue sample. More preferably the reservoir is of sufficient size to permit manipulation of the tissue sample permitting separation of the tissue cellular stratum and harvesting of those cells from the stratum suitable for grafting.

30 The apparatus may also include one or more fluid containment wells for storage of fluids such as the nutrient solution. The wells may alternatively serve as a

receptacle for a container such as a plastic or glass vial that holds the nutrient solution. Preferably, the well is capable of holding at least a 10 ml volume. Such wells permit ease of fluid application to the tissue sample. Storage of such fluids in close proximity to its site of application also provides the advantage of reducing
5 the risk of accidental leakage of the fluid and provides an easy means for accessing the fluid for accurately delivering it to either the tissue sample or the cell suspension.

In a highly preferred form of the invention the apparatus comprises a first and second member wherein:

10 (1) the first member includes:

- (a) a heating means suitable for heating an enzyme solution to a required temperature and which is capable of maintaining that solution at the desired temperature for a suitable amount of time;
- (b) a filter recess comprising a filter means capable of separating large cellular congregates from a cellular suspension;
- 15 (c) at least a fluid containment well for storage of nutrient solution;

(2) the second member forms a reservoir capable of withholding a tissue sample and nutrient solution in fluid containment; and

wherein the first member provides a seat upon which the second member may be
20 placed during manipulation of the tissue.

In a further preferred form of the invention the first member provides an storage compartment into which tools and solutions used in the above described method may be stored. Where such a compartment is provided in the apparatus the second member may provide the lid or closure to that compartment. In use the lid
25 is preferably removed from the top of the compartment and inverted. The underside of the lid preferably forms the reservoir therein enabling the second member to serve a dual purpose. Tools and solutions used in the method can be accessed from the compartment. The inverted lid may then be seated back over the compartment therein providing the reservoir for the apparatus.

The apparatus may be made from metal, plastic or any other material. Further, the container may be any size. Preferably the size of the container is only limited by its intended use and the need for sterilisation such as by the use of gamma irradiation and or ethylene oxide.

- 5 It should be appreciated that the heating means employed in the apparatus may simply constitute a heating pad on the top of the first member. There are however, attendant problems with such arrangements of the heating pad. Not least of which is the possibility of accidental spillage of the container being heated. Therefore, in an embodiment, the heating means is housed within a
- 10 recess in the first member. Also located within that recess is at least a container into which tissue may be placed for exposure to the enzyme solution. In an alternate embodiment the heating means is housed in the base of the apparatus. In such a configuration the first member contains an opening suitable for receiving a container capable of holding fluid, which opening provides access for the
- 15 container to the heating means.

In a highly preferred configuration of the apparatus a heating collar is located within a recess therein forming a heating recess in the first member within which there is located a container (eg a vial) containing lyophilised enzyme. The container is preferably held in place by at least a restraining means, which

- 20 desirably surrounds part of the upper portion of the container preventing accidental release of the container from the apparatus. In circumstances, where the apparatus is intended for single use the restraining means may be formed as an integral part of the first member, thus meaning that removal of the container may only be achieved by physically breaking the first member.
- 25 The heating means used in the apparatus is preferably controlled by circuitry permitting activation of the heating element when required. For example the heating means may be switched on by depressing the start button located, for example, on the surface of the first member. Alternately the heating means may be activated by pushing the container down with sufficient force to activate a
- 30 switch located in the base of the apparatus. A person of ordinary skill in the field will appreciate that a wide range of electronic means may be used to activate the heating unit provided in the apparatus.

Desirably the heating unit is also operably linked to a timer mechanism, which is adapted to heat the enzyme solution for a pre-defined period of time. In circumstances where the apparatus is intended for multiple uses, preferably the timer can be set to deactivate the heating element when a particular amount of 5 time is reached. At which point an alarm may activate to inform the user that the time is up. The alarm may be audible or in the form of a light display.

In an alternate more preferred form, the apparatus is designed for single use. In such instances the timer mechanism is part of the circuitry that controls the heating means. Once the heating means has been activated it heats the solution 10 for a pre-defined period of time and then self-destructs. It should be appreciated by those skilled in the art that such an the apparatus may be fitted with various monitoring means that are capable of indicating such things as: the enzyme has reached the required temperature; the amount of time that the enzyme has been in the solution for; and or the amount of time left before the circuitry self-destructs 15 etc. By way of example only, the monitoring means might consist of a series of LED's that activate when certain events occur. In a highly preferred embodiment the heating element preferably remains in the heating mode for a maximum of 45 minutes to 1 hour.

The heating means may be powered by any means known in the art. Preferably, 20 the power supply is provided by battery/batteries. In one form of the invention, the power supply is a battery or a plurality of batteries located in the base of the apparatus.

The filter recess incorporated into the apparatus may be of any size or shape that facilitates filtering of a cellular suspension. Further the recess may be adapted to 25 receive and hold at least a tube into which cell supernatant may be filtered. Preferably the recess has a conical base providing easy means to access the full volume of cell supernatant after it has been filtered.

Desirably, the third recess is designed to receive a 0.22 μ m cell filter. More preferably, the third recess can accommodate a 0.22 μ m cell filter connected to a 30 conical tube. Preferably, the tube has area/volume graduations marked on the side.

The apparatus may also include a set of tools required for skin grafting. It will be appreciated by those skilled in the art that any tools necessary for skin grafting may be included with the device. Preferably, the set of tools are sterile. As an example only, the set of tools may include a glass vessel of separation enzyme; a

5 sterile solution for suspension of the enzyme; a sterile buffered nutrient solution; scalpel; forceps; syringe; medicine dropper, cell filter; wound dressings and/or spray nozzles. In a highly preferred embodiment, the set of tools are stored in a compartment formed in the first member of the apparatus, which is covered by the second member when not in use.

10 In a highly preferred embodiment, the device is used to harvest a suspension of cells and apply the cells to a recipient site in the following manner.

An aliquot of phosphate buffered saline is mixed with a portion of lyophilised separation enzyme and placed in the heating recess. The heating means is then activated which heats the contents (ie the enzyme solution) of the container to a

15 working temperature of 37 °C preferably within 2 minutes and maintains the working temperature for at least 45 minutes. Once an operational temperature has been reach, a sample of tissue taken from a donor site is placed in the enzyme solution and incubated at the working temperature. The tissue sample is incubated for between 5 to 45 minutes. Those skilled in the art would appreciate

20 that the time taken to achieve separation of the layers of the tissue sample is dependent on the thickness of the tissue sample and the incubation temperature. Once enzymatic separation of the tissue layers is achieved, the tissue sample is removed to the reservoir and the tissue layers are separated using surgical instrument/s.

25 A carefully measured aliquot of the second solution is then withdrawn from the fluid containment well by aspiration into a syringe and then applied to the layers. The cells between the layers of tissue are scraped off and suspended by mixing with the nutrient solution. The cell suspension is then collected, preferably using a syringe and cannula.

30 The harvested suspension of cells is then passed through a cell filter located in the filter recess and the filtered suspension of cells is collected into a collection

tube also located in the filter recess. The reservoir may optionally be rinsed with a further volume of the second solution and this resulting suspension of cells also filtered and collected in the collection tube.

The filtered suspension of cells may then optionally be aspirated into a syringe
5 and applied to the recipient site.

Further features of the present invention are more fully described in the following Figures and Examples.

BRIEF DESCRIPTION OF DRAWINGS

In the figures:

10 Figure 1 illustrates a perspective view of the apparatus with the lid open and the second member in place.

Figure 2 illustrates a perspective view of the apparatus with the lid open and the second member removed and inverted.

15 Figure 3a illustrates a perspective view of the first member of the apparatus.

Figure 3b illustrates a perspective rear view of the first member of the apparatus.

Figure 4 illustrates a perspective view of the base of the apparatus.

BEST MODE(S) FOR CARRYING OUT THE INVENTION

20 It is to be understood that the following is included solely for the purposes of exemplifying the invention, and should not be understood in any way as a restriction on the broad description as set out above.

Example 1

Preparation of recipient site

25 To optimise the success of the skin graft, the wound was cleaned and assessed to be of the appropriate depth. Further, blood haemostasis was established and the wound checked for evidence of surrounding cellulitis or infection. Techniques

for preparing the area included sharp dissection, dermabrasion or laser-resurfacing.

Donor site biopsy

The donor site was chosen to appropriately match the recipient site. The donor site was infiltrated with local anaesthetic and adrenaline underneath the skin near the subcutaneous tissue. This allowed the donor site to be firm and aided in the taking a thin split-thickness biopsy. The depth of the biopsy was determined by the size of the surface area of the recipient site to be covered. Typically, the biopsy size has an expansion ratio of up to 1:10. In this case, a biopsy size of 2 cm x 2 cm was taken from the donor site.

Cell Resurfacing using the Rapid Technique

Treatment of the wound was carried out using the Re-Cell™ Rapid Technique skin replacement apparatus, which is explained in more detail in Example 2 below. The apparatus contained all the instruments, solutions, enzymes and dressings required for wound treatment.

The heating element was activated by depressing the "start button". Solution A (Calcium and magnesium free phosphate buffered saline) (10 ml) was poured from the supplied plastic vessel marked Solution A into a glass vessel, (which was already located in the heating element recess), containing the separation enzyme (lyophilised trypsin) to give a final concentration of 0.5% trypsin. The enzyme solution was then mixed together and heated to 37 °C.

The previously obtained tissue sample was then placed in the enzyme solution and incubated at 37 °C for between 10 to 15 minutes. After this time, the tissue sample was removed from enzyme solution with a pair of forceps and placed skin side down, with the epidermal side down and the dermal side up in the reservoir.

The vessel containing Solution B (nutrient media) was poured from its supplied vessel into the fluid containment well. Solution B was then aspirated from the well into a syringe and dripped from the syringe onto both layers of the biopsy.

The skin layers were separated using forceps and a scalpel. This allowed access to the zone of the dermal-epidermal junction of both surfaces. Cells were scraped from the surfaces to develop a plume of cells in the reservoir. The cells were then mixed in Solution B. The plume of cells was then drawn up into the syringe via a

5 19 gauge cannula.

The supplied filter (0.22 μ m cell filter) was mounted in the filter recess and the plume of cells in Solution B was passed through the filter. A further small amount of Solution B was then used to rinse the Petri dish and collect any remaining cells, which were also passed through the filter.

10 The resulting suspension of cells collected in the collection tube was aspirated into a syringe and a nozzle was attached to the syringe for spraying or dripping on to the wound area.

The wound was re-checked to ensure that it was clean and free of debris and that there was no evidence of bacterial contamination. Further, the wound was

15 checked to determine if haemostasis had been achieved. Once the recipient site was ready, the suspension of cells was applied to the wound surface using the nozzle.

The wound was dressed with a SurfasealTM woven nylon dressing, which was supplied with the apparatus. The healing of the wound was followed up using

20 standard protocols for skin-graft treatment.

Example 2

The embodiment shown in Figure 1 is directed to an Re-CellTM Rapid Technique skin replacement apparatus 10 for use in producing a transplantable cellular suspension of living tissue suitable for grafting to a patient.

25 As illustrated in figure 1 the apparatus includes a closure lid 12 possessing a locking mechanism 14 adapted to releasably engage a base portion 16. The locking mechanism 14 provides a means for closing the apparatus 16 when not in use. Located within the base portion 16 is a first member 18 within which there is provided an aperture 20 in which there is located a vial 22 (containing enzyme).

Adjacent the aperture there is provided an activation switch 24 capable of activating the heating means (not shown). The first member also provides a fluid containment well 26 and a filter recess 28 including a filter 29.

The aperture 20 in the first member 18 is desirably of such a diameter that it

5 allows the neck of the vial 22 to protrude through and above the first member 18. The periphery of the aperture 20 is fitted with a collar 21 which is slightly smaller than the diameter of the body of the vial 22. Thus, when in use, the vial 22 cannot be removed from the apparatus 10 as it is held in place by the collar 21 located around the periphery of the aperture 20.

10 Located adjacent to the aperture 20, fluid containment well 26 and filter recess 28 is the second member 30 which is positioned on a seat (not shown) located within a storage compartment (not shown) within the first member 18. When inverted the second member 30 forms a reservoir within which tissue manipulations may be performed. To facilitate separation of the second member 30 from the first

15 member 18 an indent 32 is provided in the side of a portion of the second member 30, which is of such a size that a person can lift the second member from the seat on which it resides in the first member 18.

Within the filter recess 28 there is located a filter 29 having a mesh therein capable of separating cellular material of greater than $0.2\mu\text{m}$ from a cell

20 supernatant.

Figure 2 provides a partially exploded perspective view of the apparatus 10 wherein the second member 30 is removed from the first member 18 and inverted. Inversion of the second member 30 reveals the sidewalls 32 of the second member 30, which form the fluid containment barrier of the reservoir and a

25 reservoir area 34 in which tissue manipulations may be performed.

Removal of the second member 30 from the first member 18 also reveals a storage compartment 36 in the first member 18 in which solutions and tools may be stored when the apparatus 10 is not in use. Within the storage compartment 36 there is located a seat 38 upon which the second member 30 may reside. The

30 seat 38 is preferably located around the periphery of the storage compartment 36

at a depth beneath the surface of the first member 18 that is equivalent to the height of the sidewalls 32 of the second member 30.

Figure 3a provides a perspective view of the first member 18 showing the storage compartment 36, the heater activating switch 24, the aperture 20, the fluid containment well 26 and filter recess 28 formed within the first member. Figure 3b provides a rear view of the first member 18 showing the filter recess 28, the fluid containment well 26, the heater activating switch 24, the aperture collar 21 and the rear wall of the storage compartment 36. As seen in this figure the filter recess has a conical base thereby providing a means for easy access to the cell suspension that is filtered into it. Located adjacent to the fluid containment well and on the opposite side of the filter containment well there is also provided a battery positioning member 40 which protrudes towards the base 16 of the apparatus (not shown) and provides a means for holding the batteries in place, which are required for activating the heating means.

5 10 15 20 25 30

Figure 4 provides a perspective view of the base 16 of the apparatus 10 showing the vial 22 located within a containment field 42. Between the containment field 42 and the vial 22 there is located a heating collar(s) 44, which surrounds the body of the vial. Adjacent the containment field there is a circuit board 46, which is held in position by circuit board containment means 48, 50 and 52. Said circuit board 46 is in electrical communications with the heating collar(s) 44 by wires 54. The circuit board is also in electrical communication via wires 58 with the heater activating switch (not shown). Adjacent the circuit board 46 there is provided a battery containment means 58, which holds 4 AA batteries in immovable position (not shown). The batteries are in electrical communication with the circuit board 46 by wires 60. When the first member 18 is fitted to the base 16 the batteries are held in place by the battery containment means 58, the battery positioning means 40 and the base of each of the fluid containment well 26 and the filter recess 28. Preferably the conical base of the filter recess 28 also protrudes between the batteries therein providing a further means for securing the batteries in immovable position.

The Claims Defining the Invention are as Follows

1. A method for preparing a cell suspension suitable for application to a patient, which method comprises the steps of:
 - (a) subjecting a tissue sample including cells suitable for grafting to a patient, to at least an enzyme suitable for dissociating cohesive pieces of tissue stratum in the sample;
 - (b) removing the tissue sample from the enzyme solution used in step (a) and harvesting in the presence of a nutrient solution cells from the tissue sample, cells suitable for grafting on to a patient wherein the nutrient solution is (i) free of xenogenic serum, (ii) capable of maintaining the viability of the cells until applied to a patient and (iii) is suitable for direct application to a region on a patient undergoing tissue grafting; and
 - (c) filtering the cellular suspension produced according to step (b) to remove large cellular conglomerates.
2. A method according to claim 1 wherein the enzyme is selected from the group consisting of trypsin, dispase or collagenase.
3. A method according to claim 1 wherein the nutrient solution is Hartmann's solution.
- 20 4. A cell suspension produced according to the method of claim 1.
5. A cell suspension according to claim 2 prepared from autologous cells.
6. A method of treating a patient in need of graft surgery, said method comprising the steps of:
 - (a) preparing an cell suspension according to the method of claim1; and
 - (b) administering the suspension directly to a region on the patient that requires a cell graft in a manner that facilitates spreading of the cell suspension in a relatively even distribution over the graft region.

7. Use of a cellular suspension suitable for grafts, which suspension is prepared according to the following steps:

5 (a) subjecting a tissue sample including cells suitable for grafting to a patient, to an enzyme suitable for dissociating cohesive pieces of the tissue stratum in the sample;

10 (b) removing the sample from the enzyme solution used in step (a) and harvesting in the presence of a nutrient solution cells from the tissue sample, which cells are suitable for grafting on to a patient wherein the nutrient solution is (i) free of xenogenic serum, (ii) capable of maintaining the viability of the cells until applied to a patient and (iii) is suitable for direct application to a region on a patient undergoing tissue grafting;

15 (c) filtering the cellular suspension produced according to step (b) to remove large cellular conglomerates;

for the preparation of medicament suitable for the treatment of tissue disorders requiring grafting.

8. An apparatus for developing a tissue regeneration solution, said apparatus comprising:

20 (a) a heating means suitable for heating an enzyme solution to a required temperature and which is capable of maintaining that solution at the desired temperature for a suitable amount of time; and

(b) a filter recess comprising a filter means capable of separating large cellular congregates from a cellular suspension.

25 9. The apparatus according to claim 8, which additionally comprises a reservoir capable of holding a tissue sample and a nutrient solution.

10. The apparatus according to claim 8, which includes one or more fluid containment wells for storage of fluids.

11. An apparatus for developing a tissue regeneration solution, comprising a first and second member wherein:

(i) the first member includes:

5 (a) a heating means suitable for heating an enzyme solution to a required temperature and which is capable of maintaining that solution at the desired temperature for a suitable amount of time;

(b) a filter recess comprising a filter means capable of separating large cellular congregates from a cellular suspension;

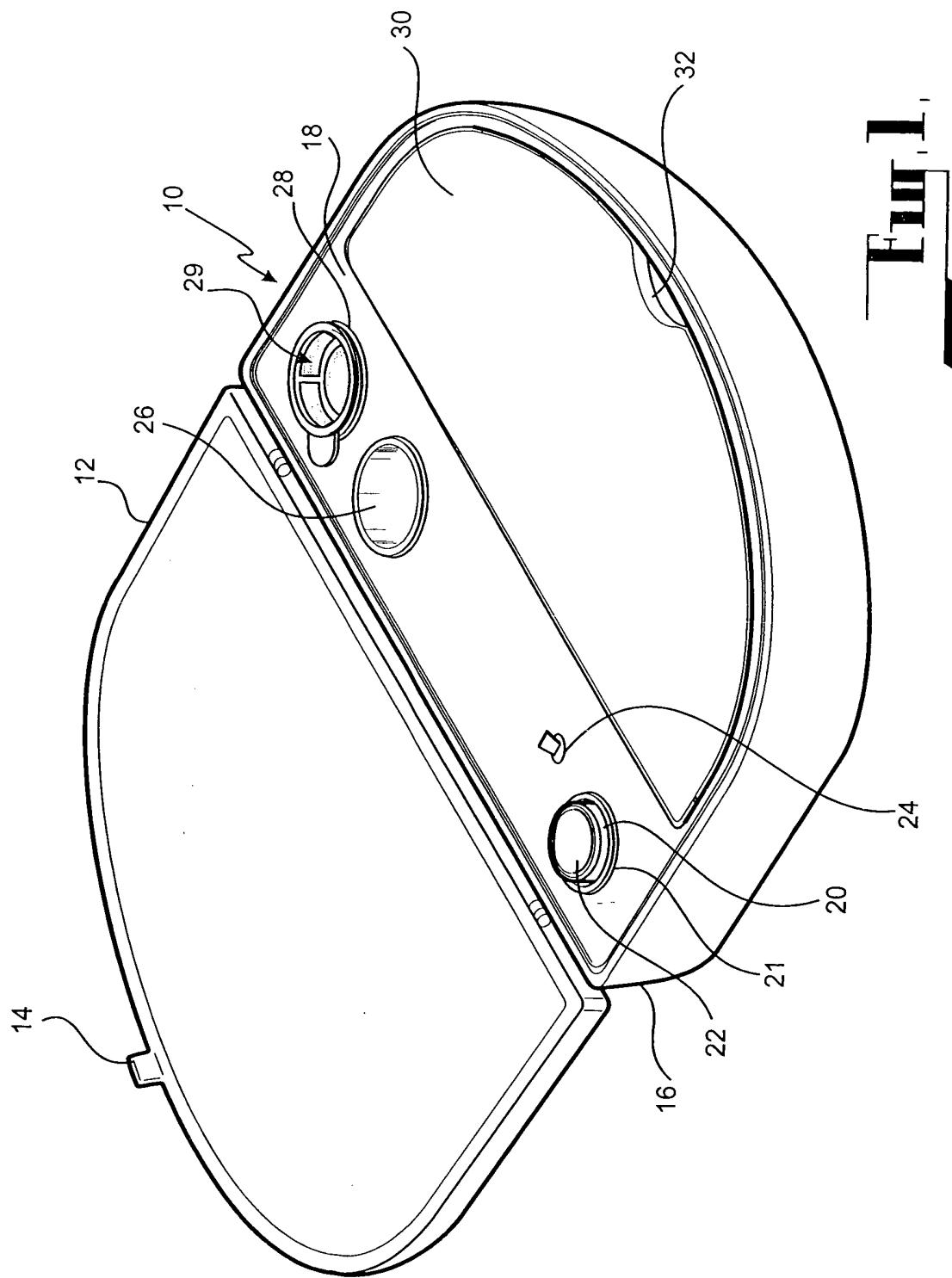
(c) at least a fluid containment well for storage of nutrient solution;

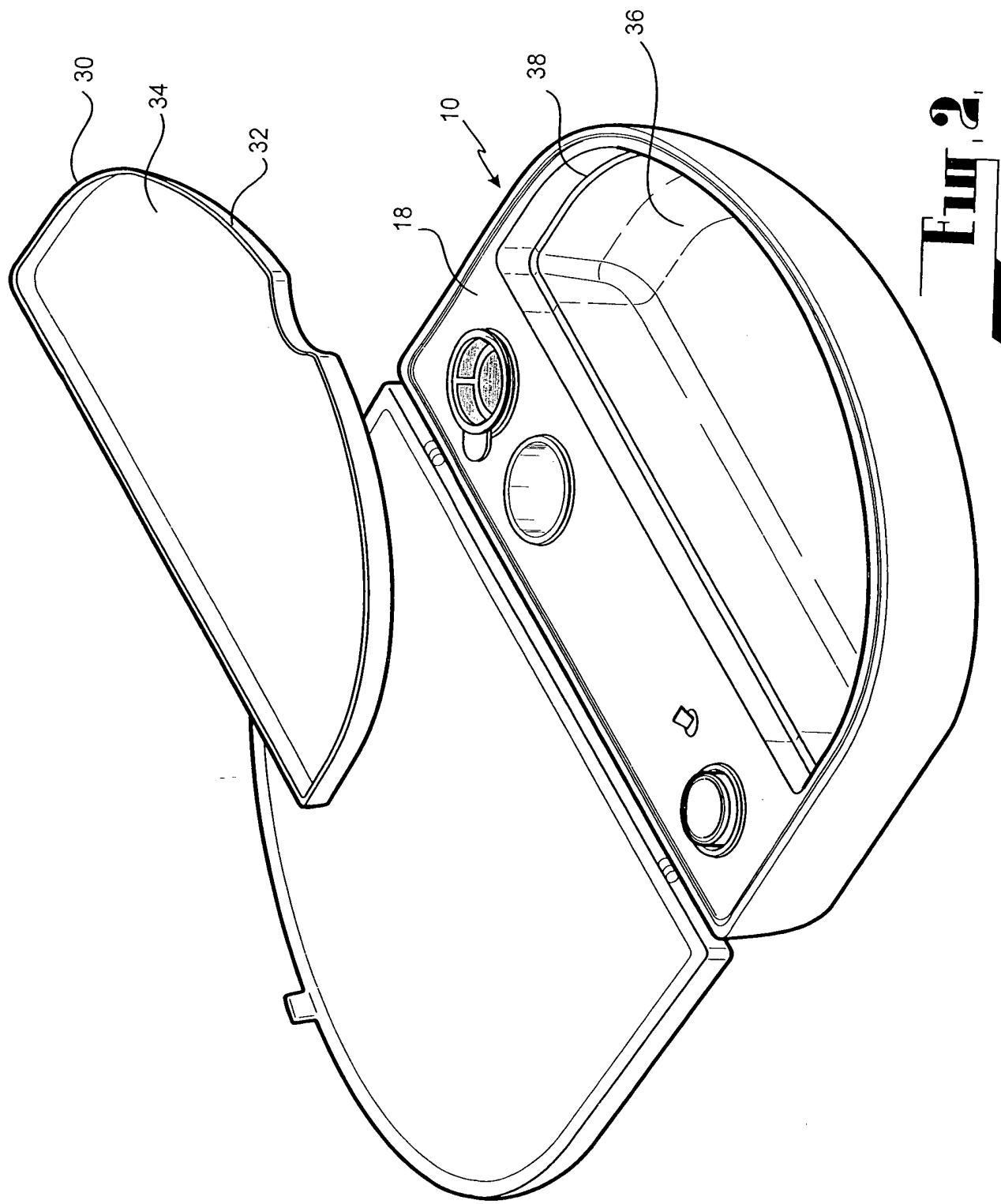
10 (ii) the second member forms a reservoir capable of withholding a tissue sample and nutrient solution in fluid containment; and

wherein the first member provides a seat upon which the second member may be placed during manipulation of the tissue.

Abstract

The present invention provides a unique method and or device suitable for producing a transplantable cellular suspension of living tissue suitable for grafting to a patient. In applying the method and or in using the device donor tissue is
5 harvested, subjected to an enzyme treatment, cells suitable for grafting back to a patient are collected and dispersed in a solution that is suitable for immediate dispersion over the recipient graft site.





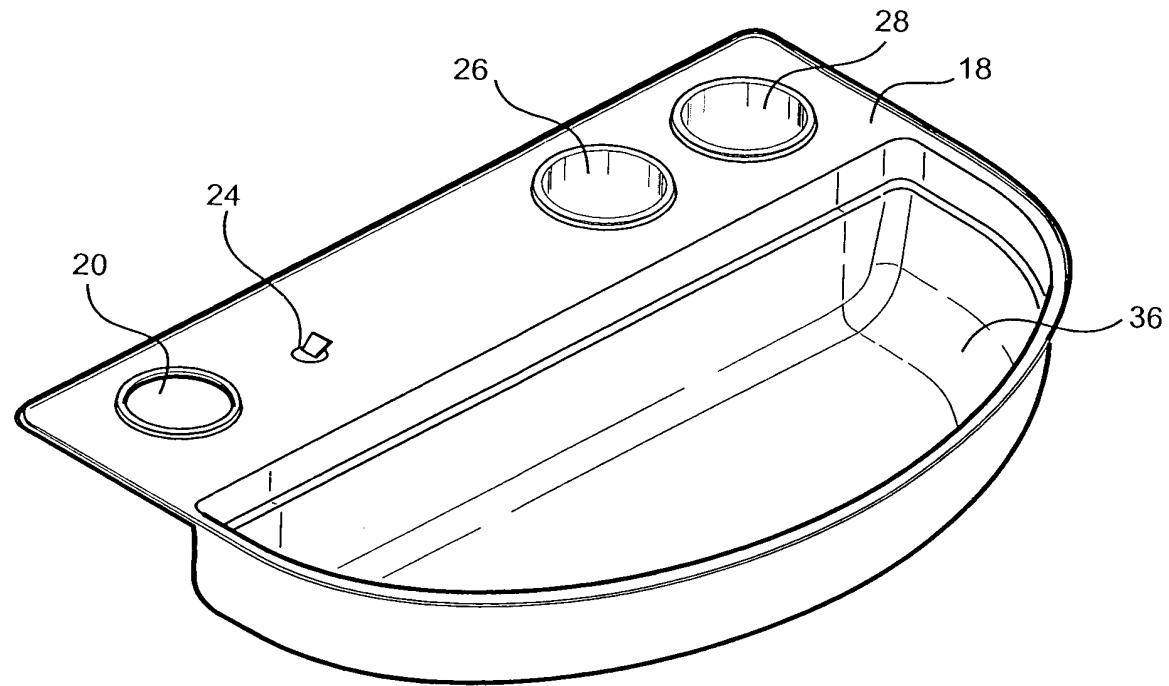


Fig. 3a.

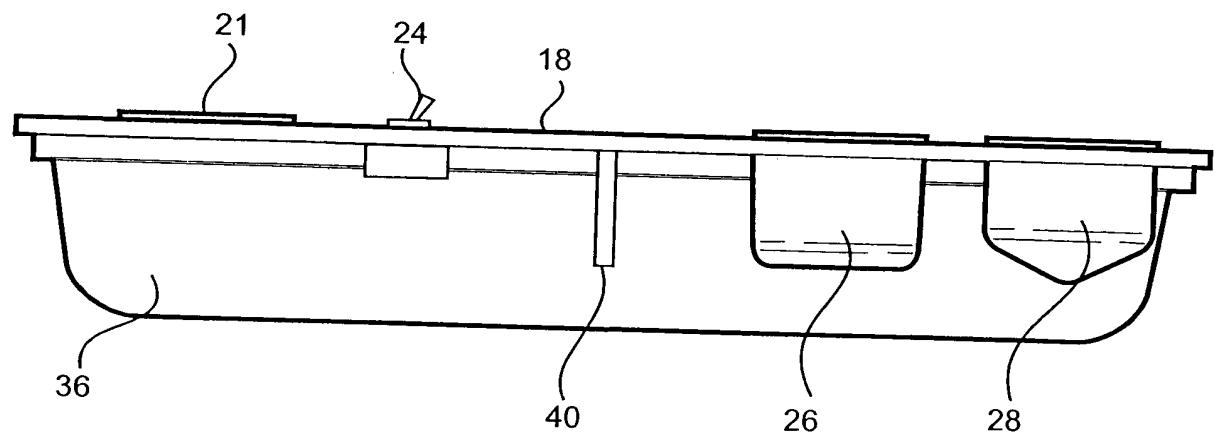


Fig. 3b.

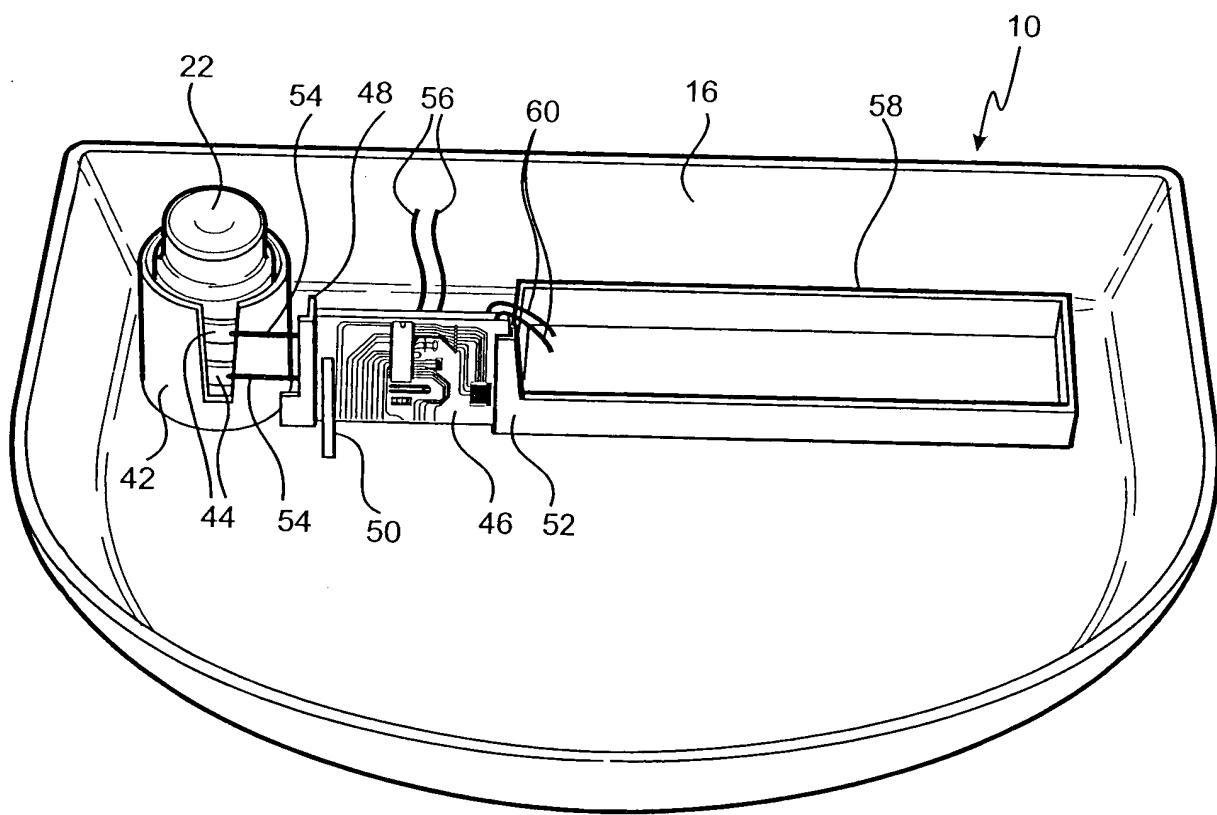


Fig. 4.